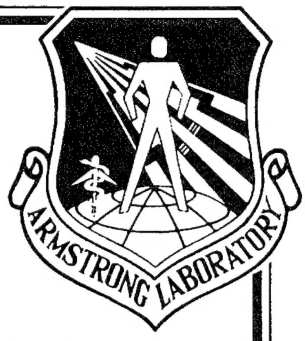
AL/OE-TR-1994-0069
VOLUME II of IV



ARMSTRONG LABORATORY

GENETIC TOXICITY EVALUATION OF 1, 3, 3-TRINITROAZETIDINE

VOLUME II: RESULTS OF MOUSE BONE MARROW MICRONUCLEUS TEST

I. J. Paika

TOXICON CORPORATION
225 WILDWOOD AVE
WOBURN, MA 01801

February 1994

FINAL REPORT FOR THE PERIOD JULY THROUGH DECEMBER 1992

AIR FORCE MATERIEL COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

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TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1994-0069

VOLUME II

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE

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Genetic Toxicity Evaluation of 1,3,3 Trinitroazetidine
Volume II: Results of Mouse Bone Marrow Micronucleus Test

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6. AUTHOR(S)
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225 Wildwood Ave
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Volume II of IV

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

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13. ABSTRACT (Maximum 200 words)

1,3,3-Trinitroazetidine (TNAZ) was dissolved in corn oil and injected intraperitonealy daily into albino Swiss mice for 3 days at doses of 40, 20, 10, 5, and 1 mg/kg. TNAZ did not induce an increased number of micronucleated cells and is considered to have a negative response. The positive control substance, mitomycin C, induced a statistically significant number of micronucleated cells, whereas the negative control substance (corn oil), did not induce increased number of micronucleated cells in the maturing erythrocytes of mouse bone marrow cells. Therefore, TNAZ is considered non-mutagenic, under the test system and conditions employed in this study.

14. SUBJECT TERMS
1,3,3-trinitroazetidine
genetic toxicity
mouse micronucleus test

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PREFACE

1,3,3-Trinitroazetidine (TNAZ) (CAS No. 97645-24-4) is a highly energetic castable explosive that is being considered by the Department of Defense for military and space applications. As a candidate replacement for select explosives, toxicity information is needed. A comprehensive literature search indicated that no information was available on the mutagenic potential of TNAZ. ManTech Environmental initiated a battery of three short-term assays that were utilized to assess the mutagenic and clastogenic potential of TNAZ. Protocols for these assays were in conformance with the Environmental Protection Agency's (Toxic Substances Control Act) Health Effects Testing Guidelines, 40 CFR, Part 798 (7-1-90 edition).

This document, Volume II of IV, serves as a final report detailing the results of the mouse bone marrow micronucleus test in the genetic toxicity evaluation of TNAZ. Volumes I and III will describe, respectively, the results of the *salmonella typhimurium* reverse mutation assay (Ames assay) and the results of gene mutation at the HGPRT locus in cultured Chinese hamster ovary cells. Volume IV will serve as a summary report presenting the pertinent findings of the three assays described in Volumes I through III.

The research described herein began in July 1992 and was completed in December 1992 by the Toxikon Corporation, Woburn, MA, under a subcontract to ManTech Environmental Technology Inc., Toxic Hazards Research Unit (THRU), and was coordinated by Darol E. Dodd, Ph.D., THRU Laboratory Director. This work was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, and was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F19). Lt Col James N. McDougal served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

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STUDY REPORT

Study Title

Rodent Bone Marrow Micronucleus Test

Company Name

ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P. O. Box 31009
Dayton, OH 45437-0009

Product Identification

1, 3, 3 - Trinitroazetidine (TNAZ)

Data Requirement

TSCA, 40 CFR, Part 798

Author

Inder J. Paika, Ph.D.

Volume Number

1 of 1

Study Completed On

December 11, 1992

Performing Laboratory

Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

Laboratory Project ID/Study Number

92G-1264

STUDY SUMMARY

The Positive control substance, Mitomycin C, induced a statistically significant number of micronucleated cells, whereas the negative control substance did not induce increased number of micronucleated cells in the maturing erythrocytes in the bone marrow cells of mice. The test substance (dissolved in corn oil) was injected intraperitonealy daily into mice for 3 days at doses of 40, 20, 10, 5, and 1 mg/kg. The test substance did not induce an increased number of micronucleated cells and is considered to have a negative response. Therefore, the test substance, 1, 3, 3-Trinitroazetidine (TNAZ) is considered non-mutagenic, under the test system and conditions employed in this study.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Company: ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31009
Dayton, OH 45437-0009

Performing Laboratory: Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

Test Substance:

Test Substance: 1, 3, 3 - Trinitroazetidine (TNAZ)

Lot/Batch #: Not Supplied

CAS/Code #: 97645-24-4

Project Officer: Darol E. Dodd 1/29/93
Darol Dodd, Ph.D. Date
ManTech Environmental
Technology, Inc.

This study was contracted to Toxikon to be conducted according to all applicable laws and regulations. Specific regulatory requirements included the current EPA (TSCA), 40 CFR, 792, Good Laboratory Practice Standards.

Study Director: Inder J. Paika 12/11/92
Inder J. Paika, Ph.D. Date:
Toxikon Corporation

QUALITY ASSURANCE STATEMENT

Company: ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31009
Dayton, OH 45437-0009

Performing Laboratory: Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

Test Substance:

Test Substance: 1, 3, 3 - Trinitroazetidine (TNAZ)

Lot/Batch #: Not Supplied

CAS/Code #: 97645-24-4

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and Management.

INSPECTIONS	QUALITY ASSURANCE INSPECTIONS	REPORTS TO MANAGEMENT	REPORTS TO STUDY DIRECTOR
SCORING	10/01/92	10/01/92	10/01/92
RAW DATA	12/10/92	12/10/92	12/10/92
FINAL REPORT	12/11/92	12/11/92	12/11/92

Signature Of Authorized Personnel:

Kathryn M. Balch, B.A.
Toxikon Quality Assurance

12/11/92
Date

TABLE OF CONTENTS

1.0 PURPOSE

The purpose of this assay is to evaluate the ability of a test substance and/or its metabolites to induce micronuclei in maturing erythrocytes of mice. This procedure detects damage of the chromosomes or mitotic apparatus caused by chemicals or other agents.

2.0 REFERENCES

This test was conducted based upon the Toxic Substance Control Act, 40 CFR, Part 798, Section 798.5395, 1991.

USP XXII, 1990.

Cihak, R. "Evaluation of benzidine by the micronucleus test." *Mutation Research* 67:383-384(1979).

Cole, R.J., Taylor, N., Cole, J., and Arlett, C.F. "Short-term tests for transplacentally active carcinogens. 1. Micronucleus formation in fetal and maternal erythroblasts." *Mutation Research* 80:141-157(1981).

Heddle, J.A., Hite, M., Kurkhart, B. Mavournin, K.,MacGregor, J.T.,Newell, G.W., and Salamone, M.F. "The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency GeneTox Program." *Mutation Research* 123:61-118(1983).

Heddle, J.A., Stuart, E. and Salomone, M.F. Handbook of Mutagencity Test Procedures, Pp 441-457. Kilbey, B.J., Legator, M. Nichols, W. and Ramei, C.; New York: Elsevier Science Publishers, (1984).

Kliesch, U., Danford, N., and Adler, I.D. "Micronucleus test and bone-marrow chromosome analysis. A comparison of 2 methods *in vivo* for evaluating chemically induced chromosomal alterations. " *Mutation Research* 80:321-332(1981).

Matter, B., and Schmid, W. "Trenimon-induced chromosomal damage in bone-marrow cells of six mammalian species, evaluated by the micronucleus test", *Mutation Research* 12:417-425(1971).

Schmid,W. "The micronucleus test." *Mutation Research* 31:9-15(1975).

Schmid, W. The micronucleus test for cytogenetic analysis. In: Chemical Mutagens, Principles and Methods for their Detection. Vol 4. Pp. 31-53, Editor, Hollaender, A.; New York: Plenum Press (1976).

3.0 COMPLIANCE

The present study conformed to all applicable laws and regulations. Specific regulatory requirements included the current TSCA, 40CFR, Part 792, - Good Laboratory Practice Standards; AAALAC, "Guide for the Care and Use of Laboratory Animals", DHHS Pub. No. (NIH) 85-23, Revised 1985; NIH (OPRR), "Public Health Service Policy on Human Care and Use of Laboratory Animals", Health Research Extension Act of 1985 (Public Law 99-158), Revised 1986; USDA, Department of Agriculture, Animal, and Plant Health Inspection Service, 9 CFR, Parts 1, 2, and 3, Animal Welfare, Final Rules 1989.

4.0 TEST SUBSTANCE

The following information was supplied by the Sponsor wherever applicable. Confidential information did not apply. The Sponsor was responsible for all test substance characterization data as specified in the GLP regulations.

Test Substance: 1, 3, 3 - Trinitroazetidine (TNAZ)
Lot/Batch #: Not Supplied by Sponsor (N/S)
CAS/Code #: 97645-24-4
Physical State: White Granular Solid
Color: White
Density: 1.84
pH: N/S
Stability: CAUTION: Class A Explosive Refer to MSDS
Solubility: Negligible in water; DMSO
Quantity: Approximately 10 grams
Source: Eglin AFB, FL 32542-5000
Storage Conditions: Refer to MSDS
Safety Precautions: Special Safety Precautions, Refer to MSDS
NOTE: Class A Explosive

5.0 IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

5.1 Historically, this assay has demonstrated to be effective in detecting clastogenic activity of chemicals. Mice are recommended, but any appropriate mammalian species may be used. The guidelines have no alternative (non-animal) methods.

5.2 Animals:
5.2.1 Source:
Healthy, not previously used albino Swiss mice (*Mus musculus*), male and female, were obtained from a registered commercial breeding laboratory (Charles River Breeding Laboratories, Wilmington, MA). At the start of the study, the animals were 7-12 weeks of age and $\geq$ 24 grams.

5.2.2 Housing:
Animals were housed in polycarbonate cages (five of each sex per cage). Hardwood chips (SANI-chips^{R}, J.P. Murphy Forest Products, Montvale, NJ) were used as bedding. The animals were housed at 68±3°F, with a relative humidity of 30-70%, a minimum of 10-13 complete air exchanges per hour, and a 12 hour light dark cycle using full spectrum fluorescent lights. The laboratory and animal rooms were maintained as a limited access facility. Animals were supplied with water and a commercial rodent chow (Agway Prolab, Waverly, NY) ad libitum. There were no known contaminants present in the feed, water, or bedding expected to interfere with the test results.

5.2.3 Quarantine:
Animals were quarantined for 8 days (Range-finding Study), 5 days (Final Study - Test and Negative Control), and 7 days (Final Study - Positive Control) prior to dose administration.

5.2.4 Animals were randomized into treatment and control groups and identified by ear punch.

6.0 ROUTE OF TEST SUBSTANCE ADMINISTRATION AND JUSTIFICATION

The test substance was administered *in vivo*, through a vehicle (corn oil) compatible with the test system. The route of administration was intraperitoneal injection (IP).

7.0 EXPERIMENTAL DESIGN

7.1 Frequency of Test Substance Administration:
The test substance and negative control substance were administered as three daily doses. The positive control substance was administered as a single dose.

7.2 Preparation of Test Substance:
The test substance was administered as received and suspended in corn oil for administration. Preparations were administered at a rate of 40 ml/kg.

7.3 Negative Control Substance:
The negative control substance was corn oil, the vehicle used for test substance preparation. The negative control (corn oil) was administered at 40 ml/kg.

7.4 Positive Control Substance:
The positive control (mitomycin C) was administered at a concentration of 0.2 mg/kg and dosed at a rate of 40 ml/kg.

7.5 Range Finding Assay:

7.5.1 The dose levels for the Micronucleus Assay were selected based on the results of the Range Finding Assay.

7.5.2 Eight treatment groups of three animals per sex were selected for dosing with the test substance. The test doses employed were 10,000, 5000, 2000, 1000, 500, 100, 10, and 1.0 mg/kg per day for 3 days. Since death immediately occured at a lower dose level, 500 mg/kg, the 10,000, 5000, 2000, and 1000 mg/kg groups were not dosed. Clinical Observations were conducted daily for 72 hours during the period of dosing. At the end of the observation period, surviving animals were euthanized by CO_2 inhalation. An attempt was made to minimize the number of animals required in the assay.

7.6 Micronucleus Assay:

7.6.1 The dose levels selected for the Micronucleus Assay were based on the Range Finding Assay. The highest dose selected (40 mg/kg) did not produce any deaths and toxicity was observed. Four lower dose levels were also selected (20, 10, 5, and 1.0 mg/kg).

7.6.2 Animals were randomized and placed into treatment groups consisting of 5 males and 5 females. The test substance was dosed by intraperitoneal injection. The test substance was administered to one treatment group per concentration required. An additional group was similarly dosed with the negative control substance. The positive control substance was administered to a single treatment group, at the concentration specified. Clinical observations were conducted daily.

7.6.3 At 72 hours, 5 males and 5 females were sacrificed from each test substance concentration and negative control substance groups after receiving 3 single doses 24 hours apart. All animals in the positive control group were sacrificed 24 hours after a single dose administration.

7.6.4 At each sacrifice interval, bone marrow slides were prepared. The animals were euthanized by cervical dislocation. Immediately after sacrifice, the femur was removed by appropriate surgical techniques. A 22g x 1" needle with a 1 cc syringe was used to push a few drops of fetal calf serum through the bone marrow cavity, flushing the bone marrow on to a clean, pre-labeled microscope slide.

A second slide, clean and pre-labeled, was inverted and placed flush to the first slide. Using a circular motion, the two slides were rubbed together until the bone marrow was evenly dispersed. The two slides were gently pulled apart and air dried. The slides were stained with SIGMA's "Accustain" Giemsa (1 part stock stain solution to 19 parts distilled water, by volume) for 5 minutes and differentiated in distilled water for 30 to 90 seconds.

8.0 EVALUATION OF CRITERIA

8.1 The test substance was considered to have a positive response in the assay if it caused a dose related response and one dose exhibited a significant increase over its concurrent negative control substance. If there was an absence of a dose response, a positive response must have at least two successive doses exhibiting a significant increase over the concurrent negative control substance.

8.2 The test substance was considered to have a negative response if it did not produce a statistically significant dose related increase in the number of micronucleated polychromatic erythrocytes or a statistically significant and reproducible positive response at any one of the test substance concentrations.

8.3 A total of 1000 polychromatic erythrocytes were scored for the presence of micronuclei. The scored elements were the number of micronucleated cells, and not the number of micronuclei. The proportion of polychromatic erythrocytes to total erythrocytes was determined.

8.4 The slides were scored blindly in order to reduce possible bias associated with the analysis. The slides were coded using random numbers.

8.5 In the negative control substance, the average number of micronucleated polychromatic erythrocytes (PCEs) per 1000 PCEs should not exceed five.

8.6 There should be a statistically significant increase in the number of micronucleated PCEs in the positive control over the negative control.

8.7 Data is to be analyzed separately for males and females. Frequency of micronucleated PCEs in each dose group will be compared to negative control using the ANOVA and/or Newman Keuls Tests. Results are considered not significant with a P value $\geq$ 0.05.

9.0 RESULTS

9.1 Range-Finding (Table IA)
Immediately after dosing, death due to the toxicity of the test substance was observed among all the animals dosed at 500 mg/kg. Death was also observed in three out of six animals dosed at 100 mg/kg immediately after dosing. The remaining three animals at the 100 mg/kg dose exhibited tremors. At 10 mg/kg, signs of tremors were observed for all animals. No signs of toxicity were observed at 1.0 mg/kg.

9.2 Final Assay (Tables 1B-1D)
For the final assay, doses were 40, 20, 10, 5.0 and 1.0 mg/kg. At the 40, 20, and 10 mg/kg dose levels, tremors were observed in all animals immediately after injection. At 5.0 mg/kg, 5 out of 10 animals exhibited tremors. The remaining 5 animals did not exhibit any signs of toxicity. No signs of toxicity were observed in any animals at the 1.0 mg/kg dose level.

9.3 Positive Control (Table 2A)
There was a statistically significant increase in the number of micronucleated polychromatic erthyrocytes in the positive control substance group compared to the negative control substance group.

9.4 Negative Control (Table 2B)
In the negative control substance, the average number of micronucleated polychromatic erythrocytes per 1000 PCEs did not exceed five.

9.5 Test Groups and Data Evaluation (Tables 2C-2G)
Each test and control group was analyzed separately for male versus female animals utilizing a Student t-test to analyze for possible sex differences. Since no statistical significance was noted in the frequency of micronuclei between males and females, the data were pooled and males and females were analyzed as a combined data set.

The frequency of micronucleated PCEs (polychromatic erythrocytes) in each dose group was compared to that of the respective negative control substance, using Tallarida, R.S. and R.B. Murray's Pharmacological Calculations Procedure, ANOVA (analysis of variance) and Newman-Keuls Test for confirmation of pairwise comparisons. All results are considered not significant at $p \geq 0.05$. There was a statistically significant increase in the number of micronucleated polychromatic erythrocytes in the positive control substance group compared to the negative control substance group, at $p \leq 0.05$.

The test substance did not produce a statistically significant dose related increase in the number of micronucleated polychromatic erythrocytes or a statistically significant and reproducible positive response at any one of the test substance concentrations.

10.0 CONCLUSION

The Positive control, Mitomycin C, induced a significantly increased number of micronucleated cells, whereas the test substance and the negative control did not induce increased number of micronucleated cells in mice. Therefore, the test substance, 1, 3, 3 - Trinitroazetidine (TNAZ), is considered to have a negative response and is non-mutagenic under the test system and conditions used in this study.

11.0 RECORDS

Original Data:	Toxikon Corporation Archives
Final Report:	Toxikon Corporation Archives
Test Substance:	All unused test substance will be returned to the Sponsor.

12.0 PAIN AND SUFFERING

There was no evidence of pain and suffering observed or reported to the Study Director during the course of the study.

13.0 ANIMAL USAGE

The Sponsor assured that to the best of their knowledge this study did not unnecessarily duplicate previous testing. An attempt was made to minimize the number of animals required in the Range Finding and Micronucleus Assays.

14.0 CONFIDENTIALITY

Statements of confidentiality were as agreed to prior to study initiation.

15.0 VERIFICATION

Protocol Signature (Toxikon):	07/27/92
Project Log Date:	08/17/92
Technical Initiation:	09/04/92
Technical Completion:	10/12/92
Final Report:	12/11/92

16.0 AUTHORIZED SIGNATURE

Inder J. Paika, Ph.D.
Study Director

12/11/92
Date

RANGE FINDING STUDY

ANIMAL WEIGHT/DOSE DATA/CLINICAL OBSERVATIONS

Animal ID #	Dose Level	Sex	Day 0 Body Weight	Dose Volume(ml)	Clinical Signs* 0hr.	4 hr.	24hr.	48hr.	72hr.
25	500 mg/kg	Male	29.0	1.16	13A	-	-	-	-
26	500 mg/kg	Male	30.7	1.23	13A	-	-	-	-
27	500 mg/kg	Male	29.5	1.18	13A	-	-	-	-
28	500 mg/kg	Female	25.1	1.00	13A	-	-	-	-
29	500 mg/kg	Female	24.3	0.97	13A	-	-	-	-
30	500 mg/kg	Female	24.9	1.00	13A	-	-	-	-
31	100 mg/kg	Male	29.7	1.19	2-I	0	0	0	0
32	100 mg/kg	Male	28.5	1.14	13A	-	-	-	-
33	100 mg/kg	Male	33.5	1.34	13A	-	-	-	-
34	100 mg/kg	Female	26.2	1.05	2-I	0	0	0	0
35	100 mg/kg	Female	26.8	1.07	13A	-	-	-	-
36	100 mg/kg	Female	26.9	1.08	2-I	0	0	0	0
37	10 mg/kg	Male	27.1	1.08	2-I	0	0	0	0
38	10 mg/kg	Male	32.2	1.29	2-I	0	0	0	0
39	10 mg/kg	Male	33.1	1.32	2-I	0	0	0	0
40	10 mg/kg	Female	24.5	0.98	2-I	0	0	0	0
41	10 mg/kg	Female	24.2	0.97	2-I	0	0	0	0
42	10 mg/kg	Female	25.7	1.03	2-I	0	0	0	0
43	1.0 mg/kg	Male	28.3	1.13	0	0	0	0	0
44	1.0 mg/kg	Male	29.6	1.18	0	0	0	0	0
45	1.0 mg/kg	Male	29.4	1.18	0	0	0	0	0
46	1.0 mg/kg	Female	25.6	1.02	0	0	0	0	0
47	1.0 mg/kg	Female	26.7	1.07	0	0	0	0	0
48	1.0 mg/kg	Female	24.9	1.00	0	0	0	0	0

* Clinical Signs (post-first injection):
13A = Death due to toxicity
0 = Normal
2-I = Tremors

ANIMAL WEIGHT/DOSE DATA/CLINICAL OBSERVATIONS
Test Article

Animal ID #	Dose Level	Sex	Day 0 Body Weight	Dose Volume(ml)	Clinical Signs* 0hr.	4 hr.	24hr.	48hr.	72hr.
1	40 mg/kg	Male	30.8	1.23	2-I	0	0	0	0
2	40 mg/kg	Male	32.5	1.30	2-I	0	0	0	0
3	40 mg/kg	Male	28.7	1.15	2-I	0	0	0	0
4	40 mg/kg	Male	29.2	1.17	2-I	0	0	0	0
5	40 mg/kg	Male	25.1	1.00	2-I	0	0	0	0
6	40 mg/kg	Female	25.8	1.03	2-I	0	0	0	0
7	40 mg/kg	Female	26.9	1.08	2-I	0	0	0	0
8	40 mg/kg	Female	26.2	1.05	2-I	0	0	0	0
9	40 mg/kg	Female	27.4	1.10	2-I	0	0	0	0
10	40 mg/kg	Female	26.3	1.05	2-I	0	0	0	0
11	20 mg/kg	Male	28.9	1.16	2-I	0	0	0	0
12	20 mg/kg	Male	27.2	1.09	2-I	0	0	0	0
13	20 mg/kg	Male	30.6	1.22	2-I	0	0	0	0
14	20 mg/kg	Male	31.9	1.28	2-I	0	0	0	0
15	20 mg/kg	Male	27.2	1.09	2-I	0	0	0	0
16	20 mg/kg	Female	23.8	0.95	2-I	0	0	0	0
17	20 mg/kg	Female	25.7	1.03	2-I	0	0	0	0
18	20 mg/kg	Female	25.6	1.02	2-I	0	0	0	0
19	20 mg/kg	Female	26.0	1.04	2-I	0	0	0	0
20	20 mg/kg	Female	29.2	1.17	2-I	0	0	0	0
21	10 mg/kg	Male	30.8	1.23	2-I	0	0	0	0
22	10 mg/kg	Male	29.4	1.18	2-I	0	0	0	0
23	10 mg/kg	Male	28.5	1.14	2-I	0	0	0	0
24	10 mg/kg	Male	30.6	1.22	2-I	0	0	0	0
25	10 mg/kg	Male	31.4	1.26	2-I	0	0	0	0
26	10 mg/kg	Female	25.2	1.01	2-I	0	0	0	0
27	10 mg/kg	Female	27.1	1.08	2-I	0	0	0	0
28	10 mg/kg	Female	26.9	1.08	2-I	0	0	0	0
29	10 mg/kg	Female	25.3	1.01	2-I	0	0	0	0
30	10 mg/kg	Female	24.1	0.96	2-I	0	0	0	0

* Clinical Signs (post-first injection):
0 = Normal
2-I = Tremors

MICRONUCLEUS ASSAY

ANIMAL WEIGHT/DOSE DATA/CLINICAL OBSERVATIONS
Test Article and Negative Control

Animal ID #	Group	Sex	Day 0 Body Weight	Dose Volume(ml)	Clinical Signs* 0hr.	4 hr.	24hr.	48hr.	72hr.
31	5 mg/kg	Male	28.3	1.13	2-I	0	0	0	0
32	5 mg/kg	Male	30.8	1.23	0	0	0	0	0
33	5 mg/kg	Male	30.2	1.21	0	0	0	0	0
34	5 mg/kg	Male	28.9	1.16	2-I	0	0	0	0
35	5 mg/kg	Male	29.7	1.19	0	0	0	0	0
36	5 mg/kg	Female	25.0	1.00	2-I	0	0	0	0
37	5 mg/kg	Female	27.6	1.10	0	0	0	0	0
38	5 mg/kg	Female	26.8	1.07	2-I	0	0	0	0
39	5 mg/kg	Female	25.3	1.01	2-I	0	0	0	0
40	5 mg/kg	Female	25.1	1.00	0	0	0	0	0
41	1 mg/kg	Male	30.3	1.21	0	0	0	0	0
42	1 mg/kg	Male	31.6	1.26	0	0	0	0	0
43	1 mg/kg	Male	30.2	1.21	0	0	0	0	0
44	1 mg/kg	Male	29.6	1.18	0	0	0	0	0
45	1 mg/kg	Male	31.4	1.26	0	0	0	0	0
46	1 mg/kg	Female	25.2	1.01	0	0	0	0	0
47	1 mg/kg	Female	25.6	1.02	0	0	0	0	0
48	1 mg/kg	Female	26.6	1.06	0	0	0	0	0
49	1 mg/kg	Female	27.4	1.10	0	0	0	0	0
50	1 mg/kg	Female	27.1	1.08	0	0	0	0	0
51	Negative	Male	32.6	1.30	0	0	0	0	0
52	Control	Male	31.0	1.24	0	0	0	0	0
53		Male	32.2	1.29	0	0	0	0	0
54		Male	29.6	1.18	0	0	0	0	0
55		Male	30.9	1.23	0	0	0	0	0
56	Negative	Female	25.4	1.02	0	0	0	0	0
57	Control	Female	26.6	1.06	0	0	0	0	0
58		Female	29.1	1.16	0	0	0	0	0
59		Female	27.1	1.08	0	0	0	0	0
60		Female	27.8	1.11	0	0	0	0	0

*Clinical Signs (post-first injection):
0 = Normal
2-I = Tremors

MICRONUCLEUS ASSAY

ANIMAL WEIGHT/DOSE DATA/CLINICAL OBSERVATIONS
Positive Control

Animal ID #	Group	Sex	Day 0 Body Weight	Dose Volume(ml)	Clinical Signs* 48 hr.	72 hr.**
61	Positive	Male	32.2	1.29	0	0
62	Control	Male	30.1	1.20	0	0
63		Male	33.9	1.36	0	0
64		Male	33.0	1.32	0	0
65		Male	29.5	1.18	0	0
66	Positive	Female	26.6	1.06	0	0
67	Control	Female	29.9	1.20	0	0
68		Female	28.8	1.15	0	0
69		Female	29.1	1.16	0	0
70		Female	27.0	1.08	0	0

*Clinical Signs (post-first injection):
0 = Normal
**Animals dosed at 48 hours and sacrificed
24 hours later at the 72 hours

TABLE 2A

ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS (BMS)

POSITIVE CONTROL SUBSTANCE

Animal #	Slide #	Sex	#PCE	#RBC	PCE/ RBC	#MNC/ SLIDE	#MNC/ 1000PCE
61	9	Male	300	120	2.50	16	
	1	Male	400	105	3.80	14	
	16	Male	250	96	2.60	15	
	30	Male	50	90	0.56	4	49
62	27	Male	350	109	3.21	13	
	32	Male	450	153	2.94	14	
	41	Male	200	62	3.23	15	42
63	17	Male	500	165	3.03	13	
	38	Male	300	96	3.13	16	
	63	Male	200	68	2.90	12	41
64	3	Male	550	193	2.85	13	
	13	Male	250	93	2.69	14	
	24	Male	200	46	4.34	13	40
65	2	Male	300	120	2.50	12	
	29	Male	550	231	2.38	14	
	18	Male	150	57	2.63	14	40
66	6	Female	400	136	2.94	15	
	4	Female	600	234	2.56	23	38
67	42	Female	300	108	2.78	14	
	55	Female	400	156	2.56	13	
	12	Female	300	96	3.13	15	42
68	51	Female	300	120	2.50	16	
	44	Female	500	190	2.63	15	
	65	Female	200	48	4.17	13	44
69	33	Female	500	205	2.44	30	
	57	Female	500	190	2.63	13	39
70	34	Female	600	222	2.70	15	
	52	Female	400	164	2.44	16	31

PCE = polychromatic erythrocytes
RBC = red blood cells
MNC = micronucleated cells

MEAN 40.60
SD$\pm$4.58

TABLE 2B

ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS (BMS)

NEGATIVE CONTROL SUBSTANCE

Animal #	Slide #	Sex	#PCE	#RBC	PCE/ RBC	#MNC/ SLIDE	#MNC/ 1000PCE
51	47	Male	400	148	2.70	1	
	70	Male	600	280	2.14	2	3
52	59	Male	500	220	2.27	1	
	100	Male	500	205	2.43	2	3
53	61	Male	400	168	2.38	2	
	94	Male	600	246	2.44	2	4
54	82	Male	400	156	2.56	2	
	127	Male	400	168	2.38	1	
	53	Male	200	76	2.63	0	3
55	71	Male	300	111	2.70	2	
	80	Male	700	238	2.94	3	5
56	111	Female	400	148	2.70	2	
	81	Female	600	216	2.78	2	4
57	101	Female	500	195	2.56	2	
	72	Female	200	76	2.63	0	
	45	Female	300	123	2.44	2	4
58	54	Female	600	234	2.56	2	
	112	Female	400	144	2.78	1	3
59	50	Female	500	200	2.50	2	
	66	Female	300	123	2.44	2	
	35	Female	200	70	2.86	0	4
60	122	Female	300	108	2.78	1	
	123	Female	200	70	2.86	0	
	62	Female	500	180	2.78	2	3

PCE = polychromatic erythrocytes
RBC = red blood cells
MNC = micronucleated cells

MEAN 3.60
SD ±0.70

TABLE 2C

ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS (BMS)

TEST SUBSTANCE - 40 mg/kg DOSE

Animal#	Slide#	Sex	#PCE	#RBC	PCE/ RBC	#MNC/ SLIDE	#MNC/ 1000PCE
1	99	Male	200	72	2.78	0	
	135	Male	300	108	2.78	1	
	147	Male	500	175	2.86	2	3
2	188	Male	500	182	2.75	2	
	193	Male	500	166	3.01	1	3
3	109	Male	200	72	2.78	0	
	181	Male	500	172	2.91	3	
	136	Male	300	108	2.78	0	3
4	154	Male	400	156	2.56	2	
	174	Male	300	106	2.83	1	
	183	Male	300	110	2.73	1	4
5	106	Male	400	162	2.47	2	
	191	Male	600	234	2.56	3	5
6	84	Female	600	204	2.94	3	
	120	Female	300	106	2.83	1	
	192	Female	100	36	2.78	0	4
7	146	Female	400	140	2.86	2	
	128	Female	300	114	2.63	1	
	87	Female	300	106	2.83	1	4
8	172	Female	500	190	2.63	4	
	160	Female	500	170	2.94	2	6
9	148	Female	500	198	2.53	2	
	196	Female	200	74	2.70	0	
	158	Female	300	102	2.94	1	3
10	184	Female	200	82	2.44	1	
	175	Female	400	162	2.47	2	
	166	Female	400	156	2.56	2	5

PCE = polychromatic erythrocytes
RBC = red blood cells
MNC = micronucleated cells

MEAN 4.00
SD ±1.05

TABLE 2D

ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS (BMS)

TEST SUBSTANCE - 20 mg/kg DOSE

Animal#	Slide#	Sex	#PCE	#RBC	PCE/ RBC	#MNC/ SLIDE	#MNC/ 1000PCE
11	182	Male	500	204	2.45	4	
	108	Male	200	76	2.63	0	
	199	Male	300	110	2.73	1	5
12	194	Male	500	204	2.45	3	
	126	Male	300	110	2.73	2	
	156	Male	200	68	2.94	1	6
13	197	Male	200	70	2.86	0	
	110	Male	500	210	2.38	4	
	152	Male	300	120	2.50	1	5
14	173	Male	200	78	2.56	0	
	168	Male	300	104	2.88	0	
	177	Male	500	186	2.69	3	3
15	144	Male	500	190	2.63	4	
	230	Male	200	74	2.70	0	
	198	Male	300	118	2.54	0	4
16	221	Female	500	201	2.49	4	
	211	Female	300	108	2.78	1	
	204	Female	200	78	2.56	0	5
17	229	Female	400	144	2.78	3	
	216	Female	300	105	2.86	1	
	219	Female	300	112	2.68	1	5
18	200	Female	300	102	2.94	0	
	207	Female	300	109	2.75	1	
	228	Female	400	152	2.63	2	3
19	201	Female	500	185	2.70	3	
	217	Female	200	72	2.78	0	
	240	Female	300	94	3.19	1	4
20	234	Female	300	108	2.78	2	
	231	Female	400	142	2.82	1	
	220	Female	300	103	2.91	1	4

PCE = polychromatic erythrocytes
RBC = red blood cells
MNC = micronucleated cells

MEAN 4.40
SD ±0.97

TABLE 2E

ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS (BMS)

TEST SUBSTANCE - 10 mg/kg

Animal#	Slide#	Sex	#PCE	#RBC	PCE/ RBC	#MNC/ SLIDE	#MNC/ 1000PCE
21	90	Male	500	180	2.78	3	
	46	Male	300	117	2.56	2	
	64	Male	200	80	2.50	0	5
22	88	Male	400	178	2.25	2	
	113	Male	600	240	2.50	3	5
23	121	Male	200	78	2.56	0	
	48	Male	500	185	2.70	3	
	73	Male	300	195	1.54	1	4
24	91	Male	500	165	3.03	2	
	56	Male	500	175	2.86	3	5
25	102	Male	300	114	2.63	2	
	58	Male	700	217	3.23	4	6
26	114	Female	500	175	2.86	3	
	60	Female	300	114	2.63	1	
	92	Female	200	68	2.94	0	4
27	103	Female	600	222	2.70	3	
	49	Female	400	164	2.44	2	5
28	107	Female	400	156	2.56	2	
	19	Female	300	102	2.94	1	
	105	Female	300	99	3.03	0	3
29	164	Female	600	228	2.63	3	
	139	Female	400	148	2.70	1	4
30	97	Female	500	175	2.86	3	
	119	Female	300	102	2.94	1	
	157	Female	200	70	2.86	0	4

PCE = polychromatic erythrocytes **MEAN** 4.50
RBC = red blood cells **SD** ±0.85
MNC = micronucleated cells

TABLE 2F

ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS (BMS)

TEST SUBSTANCE:5 mg/kg DOSE

Animal#	Slide#	Sex	#PCE	#RBC	PCE/ RBC	#MNC/ SLIDE	#MNC/ 1000PCE
31	79	Male	300	114	2.63	2	
	137	Male	300	123	2.44	1	
	176	Male	200	72	2.78	1	
	190	Male	200	68	2.94	0	4
32	150	Male	700	252	2.78	4	
	67	Male	300	108	2.78	1	5
33	133	Male	200	76	2.63	0	
	118	Male	500	185	2.70	3	
	180	Male	300	102	2.94	1	4
34	125	Male	300	111	2.70	1	
	143	Male	400	104	3.85	2	
	39	Male	300	108	2.78	1	4
35	163	Male	600	204	2.94	3	
	169	Male	400	140	2.86	1	4
36	149	Female	300	114	2.63	1	
	134	Female	400	144	2.78	2	
	179	Female	300	102	2.94	1	4
37	195	Female	500	195	2.56	2	
	68	Female	500	165	3.03	1	3
38	189	Female	500	160	3.13	2	
	145	Female	300	120	2.50	2	
	98	Female	200	78	2.56	0	4
39	167	Female	500	170	2.94	2	
	171	Female	500	180	2.78	3	5
40	93	Female	400	156	2.56	3	
	115	Female	600	252	2.38	3	6

PCE = polychromatic erythrocytes
RBC = red blood cells
MNC = micronucleated cells

MEAN 4.30
SD ±0.82

TABLE 2G

ANALYSIS OF MICRONUCLEATED CELLS IN BONE MARROW EXTRACT SMEARS (BMS)

TEST SUBSTANCE:1 mg/kg DOSE

Animal#	Slide#	Sex	#PCE	#RBC	PCE/ RBC	#MNC/ SLIDE	#MNC/ 1000PCE
41	75	Male	400	132	3.03	2	
	83	Male	300	114	2.63	1	
	130	Male	300	111	2.70	2	5
42	131	Male	500	180	2.78	2	
	159	Male	500	190	2.63	2	4
43	89	Male	200	68	2.94	0	
	140	Male	300	114	2.63	1	
	116	Male	500	170	2.94	2	3
44	95	Male	300	102	2.94	2	
	104	Male	200	78	2.56	0	
	138	Male	500	170	2.94	3	5
45	151	Male	400	136	2.94	2	
	132	Male	600	210	2.86	3	5
46	161	Female	400	134	2.99	2	
	76	Female	400	126	3.17	1	
	36	Female	200	70	2.86	1	4
47	170	Female	300	124	2.42	2	
	96	Female	300	110	2.73	1	
	117	Female	400	156	2.56	3	6
48	141	Female	200	65	3.08	0	
	186	Female	600	210	2.86	3	
	142	Female	200	70	2.86	1	4
49	37	Female	600	204	2.50	3	
	124	Female	400	160	2.50	2	5
50	162	Female	500	195	2.56	3	
	77	Female	500	200	2.50	2	5

PCE = polychromatic erythrocytes
RBC = red blood cells
MNC = micronucleated cells

MEAN 4.60
SD ±0.84

PROTOCOL AMENDMENT 92G-1264.1

Company: ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31009
Dayton, OH 45437-0009

Performing Laboratory: Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

Test Substance:

Test Substance: 1, 3, 3 - Trinitroazetidine (TNAZ)

Lot/Batch #: Not Supplied

CAS/Code #: 97645-24-4

Amendments:

1. Protocol section 8.1 stated that the test substance, negative control, and positive control would be dosed at a rate of 10 ml/kg. Due to the viscosity of the test solution, the rate was increased to 40 ml/kg in order to get the preparations into a syringe. The controls were likewise increased.

2. Protocol section 8.6.1 stated that two lower dose levels would be used below the high dose. This was a typographical error. The protocol should indicate four lower doses were utilized below the high dose.

3. Protocol section 8.6.1 states that the four lower dose groups would be one-half, one-third, one-fourth and one-eighth the high dose level. Based upon the Range Finding and in order to find an accurate dose response, these dose groups were changed to four lower dose groups which were 1/2, 1/4, 1/8, and 1/40 of the highest dose selected which was 40 mg/kg.

4. Protocol section 9.2.5 states that the data will be analyzed at each dose level compared to the negative control group utilizing a Student's T-test. The T-test was only utilized to test for sex differences at each dose group. The data were pooled and all groups were analyzed utilizing analysis of variance (ANOVA) since there were multiple groups. The Neuman Keuls test for pair-wise group comparisons was also utilized.

5. Protocol section 8.5.3 stated that a negative control group would be employed for the Range Finding Study. In an attempt to minimize the number of animals required for the study (See

Protocol section 8.5.1), a negative control group was deemed unnecessary by the Study Director

6. Protocol sections 8.6.2 and 8.6.3 call for the use of three treatment groups (5 males/5 females) per dose level of test article (5 different dose levels) and negative control article (1 dose level) to be sacrificed at 24, 48, and 72 hours. The sponsor requested (via a Statement of Work) that a "single sacrifice time up to but not over 24 hours after last test article administration" be performed instead. Therefore, a total of 60 animals were used, 5 males/5 females per test article dose level (5) and negative control dose level (1). These animals were sacrificed 24 hours after the last dose, 72 hours after the initial dosing of the animals (test article and negative control article only). The positive control group was dosed at 48 hours and sacrificed at 72 hours in order to sacrifice all groups at the same time.

7. Section 2.1 of the protocol states that the Sponsor's address would be:

ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31008
Dayton, OH 45431-0009

Per Sponsor's request, the address has been corrected to:

ManTech Environmental Technology, Inc.
Toxic Hazards Research Unit
P.O. Box 31009
Dayton, OH 45437-0009

Signature of Authorized Personnel:

Inder J. Paika
Study Director

12/11/92
Date

Darol Dodd, Ph.D.
ManTech Environmental Technology, Inc.

1/29/93
Date

U. S. Government Printing Office 1996 750-071/00171